understanding the principles of protein interactions. Experimental studies like alanine scanning mutagenesis require significant effort; therefore, there is a need for computational methods to predict hot spots in protein interfaces. We present a new efficient method to determine computational hot spots based on sequence conservation and solvent accessibility of the interface residues (Tuncbag et al.; Guney et al.). The predicted hot spots are observed to correlate with the experimental hot spots with an accuracy of 71% and a positive predictive value of 79%. Several machine learning methods (SVM, Decision Trees and Decision Lists) are also applied to predict hot spots and compared to our method. The results reveal that our empirical approach performs better. We observe that both the change in accessible surface area upon complexation and residue accessibility in the complex forms improve detection of hot spots. Predicted computational hot spots for all protein interfaces (49512 interfaces as of 2006) are available at HotSprint database. HotSprint (a database of computational hot spots in protein interfaces) can be accessed at http://prism.ccbb.ku.edu.tr/hotsprint.

Guney E, Tuncbag N, Keskin O, Gursoy A: HotSprint: database of computational hot spots in protein interfaces Nucleic Acids Res 2008, 36(Database issue):D662-666.

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3360-Pos Board B407

Ancestral Sequence Reconstruction and Homology Modeling Link Temperature Adaptation and Conservation of Function With Sequence Evolution in Parvalbumin

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Temperature is a key factor influencing protein structure and function in poikilotherms. Previous studies of enzymes have shown that orthologs from species acclimated to different thermal niches can maintain a relatively similar level of function at those species' respective physiological temperatures. In some well-characterized enzymes, this conservation of function is correlated with differences in primary structure that lie outside active sites. Information gained from thermal adaptation studies of enzymes can be extended to non-catalytic proteins, which are less thoroughly examined, through the study of parvalbumin structure and function. Parvalbumins are intracellular calcium-binding proteins of the EF-hand type that are thought to function in muscle cells as calcium sinks permitting more rapid unloading of troponin-C, leading to more rapid contraction/relaxation cycles. Parvalbumins contain two functional, highly conserved binding sites and one non-functional site, the AB domain, thought to be an important area of modulation for cation binding. Parvalbumins from teleosts of the sub-order Notothenioid and the unrelated Arctic cod, Boreogadus saida, have converged on a common phenotype. That is, they show similar thermal sensitivity patterns of calcium binding. To explore the underlying structural basis of this similarity in phenotype, we have used ancestral sequence reconstruction combined with homology modeling to identify potential changes in primary structure that have allowed parvalbumins from these disparately related groups of fish to function similarly in their polar habitats. For instance, an Asn to Cys change at position 26 (located in the AB domain) in the evolution of B. saida parvalbumin may lead to the loss of a hydrogen bond in the B-helix. This may provide the change in tertiary structure needed for this parvalbumin to function at polar temperatures.

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Evolutionary Analyses Of KCNQ1 And HERG Voltage-gated Potassium Channel Sequences Reveal Location-specific Susceptibility And Augmented Chemical Severities Of Arrhythmogenic Mutations

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Mutations in HERG and KCNQ1 potassium channels have been associated with Long QT syndrome and atrial fibrillation, and more recently with sudden infant death syndrome and sudden unexplained death. In other proteins, disease-associated amino acid mutations have been analyzed according to the chemical severity of the changes and the locations of the altered amino acids according to their conservation over metazoan evolution. Here, we present the first such analysis of arrhythmia-associated mutations (AAMs) in the HERG and KCNQ1 potassium channels. Using evolutionary analyses, AAMs in HERG and KCNQ1 were preferentially found at evolutionarily conserved sites and unevenly distributed among functionally conserved domains. Non-synonymous single nucleotide polymorphisms (nsSNPs) are under-represented at evolutionarily conserved sites in HERG, but distribute randomly in KCNQ1. AAMs are chemically more severe, according to Grantham's Scale, than changes observed in evolution and their severity correlates with the expected chemical severity of the involved codon. Expected chemical severity of a given amino acid also correlates with its relative contribution to arrhythmias. At evolutionarily variable sites, the chemical severity of the changes is also correlated with the expected chemical severity of the involved codon. Unlike nsSNPs, AAMs preferentially locate to evolutionarily conserved, and functionally important, sites and regions within HERG and KCNQ1, and are chemically more severe than changes which occur in evolution. Expected chemical severity may contribute to the overrepresentation of certain residues in AAMs, as well as to evolutionary change.

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Evolution of Mammalian HCN Channels - Positive Darwinian Selection Identified at the Molecular Level

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HCN channels are important for regulating spontaneous electrical activity and membrane potential in excitable cells. We hypothesize that the four mammalian HCN genes were established by duplication after the divergence of urochordates and before the divergence of fish from the tetrapod lineage. A question in which we are interested is how did the differences in structure and function among the four mammalian channels arise? These differences are due to changes in primary sequence that have occurred since duplication. At the molecular level, changes in DNA sequence over the course of evolution may be identified based upon the Neutral Theory of Molecular Evolution, which states that the majority of changes in DNA are neutral. Neutrality can be estimated directly by comparing the number of non-synonymous changes (dN) and synonymous changes (dS) at the DNA level over a given period of evolutionary time. Neutrality implies that dN and dS are equal (dN/dS=1). A ratio less than one implies purifying selection, whereas a ratio of more than one implies positive selection. Here, we use phylogenetic and statistical analyses of mammalian HCN sequences to identify positive selection among the four mammalian HCN isoforms. We find that the HCN2 isoform yields a very high value for dN/dS ($>>1$), with strong statistical support. Further analysis of HCN2 uncovered a number of specific sites that have undergone positive selection, including an unusual triplet of residues in the outer pore. Our results suggest that positive selection has contributed to differences in structure and function between HCN2 and the other three isoforms.

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Genomic Identification of Transmembrane β-Barrels (TMBBs)

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Transmembrane beta-barrels (TMBBs) are a special structural class of proteins predominately found in the outer membranes of Gram-negative bacteria, mitochondria, and chloroplasts. It is estimated that 2-3% of a bacterial genome encodes TMBBs, yet less than 40 non-redundant structures have been solved. It would be highly advantageous to have methods to rapidly identify TMBBs from increasingly available genomic databases. A prediction algorithm proposed by Wimley in 2002 was based on the physicochemical properties of TMBBs of known structure. This method used relative amino acid abundances to predict the position of beta-strands and beta-hairpins, which are the major structural subunits of TMBBs, and a mathematical simplification of the topology prediction data called a beta-barrel score. To test the accuracy of this algorithm we scored proteins from a non-redundant database of protein sequences from the Protein Data Bank (NRPDB). The results revealed that the algorithm's ability to discriminate true TMBBs from other proteins, while strong, could be significantly improved. First, we updated the relative amino acid abundances to include the latest structural information. Second, we altered the beta-strand prediction method to account for the fact that certain amino acids have a higher propensity to situate near the lipid/water interface than in the hydrophobic core of the bilayer. Third, we adjusted the calculation of the beta-barrel score to address the lowered beta-hairpin density of larger TMBBs such as BtuB. We reanalyzed the NRPDB and the modifications resulted in a 5-fold decrease in the number of false positives, many of which are either non-bacterial proteins or from Gram-positive organisms. We will use this method to analyze the available genomes of Gram-negative bacteria and the results, along with the signal peptide predictions of SignalP (Bendtsen, et al. 2004) will be deposited into a publicly available database.

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Predicting Binding Sites of EH1-like Motifs from Their Amino Acid Sequences

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Bioinformatics and computer modeling were used to predict binding sites of engrailed homology-1 (eh1) -like motifs from their amino acid sequences. According to previous studies, an eh1 motif provides its transcriptional function